EFFECTS OF ICHTHYOPHONUS HOFERI ON CONDITION INDICES AND BLOOD CHEMISTRY OF EXPERIMENTALLY INFECTED RAINBOW TROUT (ONCORHYNCHUS MYKISS)

Authors: Rand, Thomas G., and Cone, David K.

Source: Journal of Wildlife Diseases, 26(3) : 323-328

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-26.3.323

Journal of Wildlife Diseases, 26(3), 1990, pp. 323–328

EFFECTS OF *ICHTHYOPHONUS HOFERI* ON CONDITION INDICES AND BLOOD CHEMISTRY OF EXPERIMENTALLY INFECTED RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

Thomas G. Rand and David K. Cone
Biology Department, Saint Mary's University, Halifax, Nova Scotia, Canada B3H 3C3

ABSTRACT: Body condition, hepatosomatic index and blood chemistry of *Oncorhynchus mykiss* experimentally infected with a tissue dwelling fish pathogenic fungus, *Ichthyophonus hoferi*, were monitored over a 6 wk period. This was to determine whether the infection constituted a stress manifest by changes in the hypothalamic-pituitary interrenal axis, and especially plasma cortisol levels. Infection caused anaemia and leucopenia but did not change the condition, hepatosomatic indices, or plasma chloride, cholesterol, cortisol, creatinine, glucose, osmolarity, potassium, total protein, sodium and T4. It is suggested that increased cortisol levels may not be a normal component of the stress response of fish to disease caused by invasive infectious agents.

Key words: Rainbow trout, *Oncorhynchus mykiss*, *Ichthyophonus hoferi*, ichthyophonosis, pathology, blood chemistry, stress response, experimental study.

INTRODUCTION

It has been postulated (Pickering, 1981) that stress on the endocrine system, particularly cortisol production, may be an important factor underlying the course of infectious diseases in fish. Cortisol, for example, induces lymphocytopenia (McLeay, 1973; Pickering and Pottinger, 1985) and increases the susceptibility of fish to fungal (Donaldson, 1981; Pickering and Durston, 1983; Pickering and Pottinger, 1985; Roth, 1972), bacterial (Donaldson, 1981; Pickering and Durston, 1983; Pickering and Pottinger, 1985) and parasitic (Woo et al., 1987) infections. However, in a detailed study of changes in the blood chemistry of *Oncorhynchus mykiss* experimentally infected with the hemoflagellate, *Cryptobia salmositica*, Laidley et al. (1988) did not find proof of involvement of the endocrine system in cryptobiosis. The present study essentially duplicates the work of Laidley et al. (1988) with a different parasite system. This was to confirm that lack of involvement of the endocrine system is normal for teleosts. We monitor condition indices, cortisol production and other blood parameters during the course of experimental infection of the fungus *Ichthyophonus hoferi* in rainbow trout. This organism causes a chronic, systemic disease with formation of granulomatous lesions in internal organs, especially the kidneys, spleen, liver and heart (McVicar, 1982).

MATERIALS AND METHODS

Fresh *Ichthyophonus hoferi* was obtained from infected tissues of yellowtail flounder (*Limanda ferruginea*) collected from the Nova Scotian Shelf in March, 1988. The pathogen was aseptically excised from infected flounder tissues and placed on petri dishes containing 10 ml of Earle's fish saline agar (EFSA) consisting of 6.8 g NaCl; 0.2 g CaCl; 0.1 g $MgSO_4$; 0.4 g KCl; 0.14 g $NaCO_3$; 2.2 g $NaH_2PO_4.H_2O$; 1.0 g glucose; 14 g BACTO™ agar; 0.5 g streptomycin and 0.5 g penicillin G and 100 ml bovine serum in 1.0 liter distilled water. The isolates were then incubated at 10 C for 2 wk before they were used.

Sixty rainbow trout weighing between 105 and 236 g were obtained from the Kinston Peninsula Hatchery (New Brunswick, Canada N1G 5A1). Fish were equally allocated to four 80 l fibreglass tanks with a continuous source of well aerated freshwater (12 to 14 C) and maintained under a natural photoperiod. After 6 wk of acclimatization, each fish in two of the four tanks was inoculated intraperitoneally with a 1 ml suspension of *I. hoferi* spores and thallus tissues in Earle's fish saline (Humason, 1972) using a 20 gauge needle under light MS 222 anaesthesia (Sigma Chemical Co., St. Louis, Missouri 63178, USA). The inoculum was prepared by aseptically removing spores and thalli from the 2-wk-old cultures of *I. hoferi* from flounder tissues on

TABLE 1. Changes in the number of rainbow trout experimentally infected with *Ichthyophonus hoferi* showing gross clinical signs of disease.

Week post infection	Number trout exhibiting clinical signs (%)	Number of lesions	Location of lesions[b]
1	0 (0)	0	—
2	1 (20)	0[a]	—
3	3 (60)	1–3	L, S
4	2 (40)	1–3	L, S, K
5	4 (80)	1–12	L, S, K
6	5 (100)	7–23	L, S, K

[a] Asymptomatic infection (spores in kidneys).
[b] L, Liver; S, Spleen; K, Kidneys.

EFSM. These were added to sterile Earle's fish saline (Humason, 1972) and diluted to achieve a final concentration of approximately 1,000 fungal units/ml saline.

Five control and five infected fish were necropsied starting at 0600 hr each week on weeks one to five post-inoculation (PI). The fish were dip netted from each tank and killed using a MS 222 overdose at a concentration (1.5 g/liter) (within approximately 30 sec) to prevent stress related elevations in cortisol (Barton et al., 1980) and T_4 (Brown et al., 1978).

Standard body length (cm), and body and liver weights (g) were determined for each fish. The condition and hepatosomatic indices of all the fish were determined according to Laidley et al. (1988): Condition index = Fish weight (g) (100)/fish length $(cm)^3$; Hepatosomatic index = Liver weight (g) (100)/fish weight (g).

Blood samples were taken by cardiac puncture and collected in both heparinized hematocrit tubes (Dade Division American Hospital Supply Corporation, Miami, Florida 33152, USA) (for packed blood cell volume) and heparinized Eppendorf centrifuge tubes (Sarstedt, St. Laurent, Quebec, Canada H4S 1E9) (for blood cell counts, blood smears, blood plasma chemistry and hormone studies). Samples for packed cell volumes were centrifuged for 3 min in a Sorvall hematocrit centrifuge (Fisher Scientific Limited, Terminal, Ottawa, Ontario, Canada K1G 4A9) and haematocrit levels were determined.

Erythrocyte and leucocyte counts for each necropsied fish were determined in duplicates using the methods outlined in the Hausser and Levy-Hausser Hy-Lite Corpuscle counting chamber instruction pamphlet (Hausser Scientific, Township Line Road, Blue Bell, Pennsylvania 19422, USA).

Blood smears from each fish were made in duplicate. These were fixed with absolute methanol and one stained with Giemsa stain for blood cells and fungi, the other with PAS (Humason 1972) for microorganisms and then examined using a compound microscope.

Blood samples for plasma chemical studies were centrifuged in the Eppendorf centrifuge tubes at 10,000 × *g* for 3 min using the Sorval table top centrifuge (Fisher Scientific Limited). The plasma was then separated from the cells and stored at −80 C until it could be analyzed, within 6 wk of collection. Plasma chloride, cholesterol, creatinine, glucose, sodium, potassium chloride, osmolarity, glucose, creatinine, total osmolarity, potassium, total protein and sodium levels were determined using a Dacos Analyzer (Coulter Electronics Inc., Hialiah, Florida 33010, USA). Plasma T_4 and cortisol concentrations were measured using highly specific radioimmunoassays supplied by Diagnostic Products Laboratory (DPC, Los Angeles, California 90045, USA).

Differences among means from infected and control rainbow trout were tested by use of a two way ANOVA (SPSS Inc., Chicago, Illinois 60611, USA). The data are presented as arithmetic mean plus the standard deviation in parentheses.

RESULTS

The number of rainbow trout showing signs of ichthyophonosis after interperitoneal inoculation with *I. hoferi* increased from none after the end of week one to 100% at the end of the experiment (Table 1). None of the control fish showed signs of the disease.

The highest numbers of lesions were associated with the liver, spleen and kidneys of infected trout and ranged from one to a few focal, creamy white patches (0.1 to 0.5 mm in diameter) at the end of week two, to many confluent patches (0.3 to 8.1 mm in diameter) at the end of week six PI.

There were no significant differences in the mean condition indices nor hepatosomatic indices between infected and uninfected fishes over the course of the experiment (Table 2). Mean hematocrit levels of infected fish declined markedly from week one to two PI and were significantly lower than those of uninfected fish from the end of week two to week six. Hematocrit levels of infected fish were lowest at the end of week six (Fig. 1).

TABLE 2. Condition and hepatosomatic indices of rainbow trout experimentally infected with *Ichthyophonus hoferi*.

Parameter	Week post infection					
	1	2	3	4	5	6
Condition factor[a]	1.2 (0.08)[b]	1.2 (0.07)	1.3 (0.11)	1.2 (0.11)	1.2 (0.23)	1.1 (0.03)
	1.2 (0.03)	1.1 (0.06)	1.1 (0.06)	1.2 (0.05)	1.1 (0.08)	1.1 (0.13)
Hepatosomatic[a] index	1.1 (0.23)	1.5 (0.30)	1.9 (0.19)	1.4 (0.32)	1.4 (0.46)	1.4 (0.18)
	1.4 (0.26)	1.9 (0.28)	1.6 (0.31)	1.5 (0.13)	1.5 (0.74)	1.8 (0.56)

[a] Controls followed by experimentally infected rainbow trout; $n = 5$ for each group.
[b] Mean with (standard deviation).

Mean erythrocyte levels in infected fish declined markedly from 1.4×10^6 at the end of week one to a maximal low of about 1.35×10^6 at the end of week two PI. They were always lower than those in uninfected fish but highly significantly so ($P < 0.005$) only at the end of weeks two, five and six (Fig. 2). In contrast, leucocyte counts in infected fish increased markedly between weeks one and two and were significantly higher ($P < 0.05$) than those of the uninfected fish from weeks five to six (Fig. 3).

Plasma glucose levels declined markedly in infected fish from the end of week one to its lowest at the end of week five. However, they were not significantly different from those in the uninfected fish (Table 3). Similarly, plasma T_4 concentrations fluctuated markedly in both the infected and uninfected fish but at no time were the mean concentrations between both groups of fish significantly different (Table 3). Both plasma cholesterol and protein concentrations in infected fish were generally lower than they were in the uninfected fishes. Again, differences were not statistically significant (Table 3).

The plasma osmolarity, and creatinine, chloride, potassium and sodium concentrations in both the infected and control fish fluctuated similarly throughout the experiment; there were no significant differences in any of these parameters between infected and control fish at any time period (Table 3).

Plasma cortisol concentrations of infected fish increased from week one to a maximal high by the end of week three PI. However, at no point in the course of the infections did levels in infected and control fish differ significantly (Table 3).

DISCUSSION

There was no significant effect of *I. hoferi* on the blood plasma chemistry of infected rainbow trout. Parameters studied were similar to those reported for salmonids by other investigators (Groman and Miller, 1987; Hille, 1982; Laidley, et al., 1988; Lockhart and Metner, 1984; Roberts, 1978; Soivio et al., 1977). The absence of elevated cortisol levels specifically suggests that there is only a limited stress response in the infective period of *I. hoferi* in rainbow trout and that it does not involve changes in the hypothalamic-pituitary interrenal axis by which stressors bring out corticosteroid stress responses in fish (Donaldson, 1981).

Infected fish did show significantly reduced hematocrit and erythrocyte counts. Numerous contributing factors are known to result in anemia, including hemodilution, bacteria producing hemolysins, hemorrhage, and renal and splenic diseases, among others (Wedemeyer and McLeay, 1981; Laidley et al., 1988; Roberts, 1978). Interestingly, Laidley et al. (1988) also found that infection of rainbow trout with *C. salmositica* resulted in anemia, a condition they attributed to hemolysis and hemodilution caused by infection. In the present study we suggest that the anemia

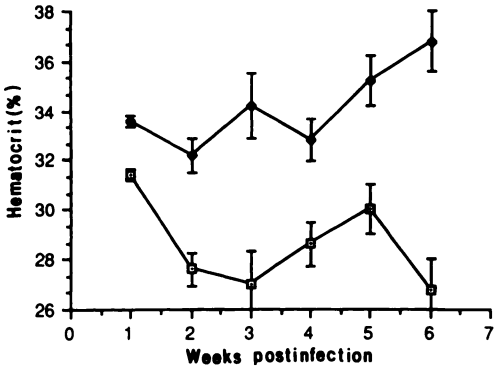

FIGURE 1. Temporal changes in the mean hematocrit levels (%) in control (-◆-) and *Ichthyophonus hoferi*-infected (-⊡-) rainbow trout. Vertical bars represent one standard error ($P < 0.01$).

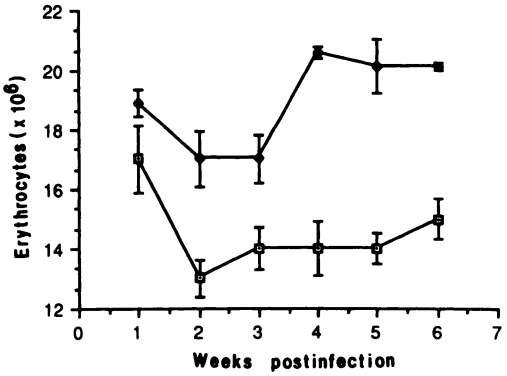

FIGURE 2. Temporal changes in mean erythrocyte abundance in control (-◆-) and *Ichthyophonus hoferi*-infected (-⊡-) rainbow trout. Vertical bars represent one standard error ($P < 0.005$).

in the trout infected with *I. hoferi* is due to the failure of hemopoietic tissues, a condition known as hypoplastic anemia (Roberts, 1978). We propose this because of the pathology associated with the high numbers of resting spores in kidneys, spleen, heart and liver, all of which have a role in erythropoiesis (Groman, 1984). Also, it is known that granulomatous conditions affecting kidneys and spleen can cause hypoplastic anemia (Roberts, 1978).

The infection with *I. hoferi* also led to a steady increase in the numbers of circulating leucocytes. This supports the studies of Amlacker (1965), Dorier and Degrange (1961) and McVicar and McLay (1985) who reported increased leucocytic activity in the early experimental infections of *I. hoferi*. The present study demonstrates that the leucopenia is a generalized host response and supports McVicar (1982) who showed that there is cell-mediated response to the wall of the resting spore. The antibody production was against spores, thalli surfaces and growing thalli tips.

The responses of body and blood plasma constituents in rainbow trout experimentally infected with *C. salmositica* and *I. hoferi* were quite different. The most apparent differences were the statistically significant hepatomegaly, and depressed plasma T_4, protein and glucose concentrations in fish infected with *C. salmositica* and these were found to be lacking in fish infected with *I. hoferi*.

It has been presumed that disease constitutes an environmental stressor in fish (Wedemeyer and McLeay, 1981). Because certain environmental stressors, including handling, confinement, transport, stocking, hypoxia, and toxic and medical substances, have been shown to result in prolonged elevations of plasma cortisol (Barton et al., 1980; Hille, 1982; Pickering and Stewart, 1984; Redgate, 1974; Simpson, 1975/1976; Tomasso et al., 1981), it was thought that infectious diseases also may

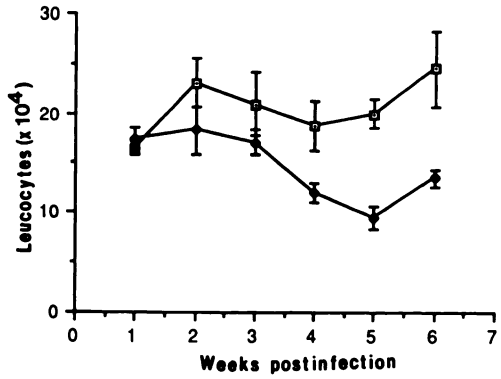

FIGURE 3. Temporal changes in mean leucocyte abundance in control (-◆-) and *Ichthyophonus hoferi*-infected (-⊡-) rainbow trout. Vertical bars represent one standard error ($P < 0.05$).

TABLE 3. Blood plasma constituents (nmol liter^{-1}) in rainbow trout experimentally infected with *Ichthyophonus hoferi*.

Parameter	1	2	3	4	5	6
Cholesterol[a]	4.6 (1.2)[b]	5.0 (0.6)	6.0 (2.1)	5.9 (1.8)	6.3 (1.4)	3.9 (1.6)
	3.6 (1.8)	4.2 (1.3)	4.8 (2.2)	6.3 (1.2)	4.4 (1.1)	4.8 (0.7)
Chloride	133 (11.3)	137 (9.1)	131 (10.2)	137 (11.0)	138 (14.3)	138 (6.6)
	139 (12.2)	135 (11.6)	138 (15.8)	137 (9.3)	140 (7.5)	129 (14.2)
Cortisol	92.8 (8.7)	85.4 (9.1)	81.4 (11.9)	79.2 (12.7)	106 (31.3)	98.4 (17.4)
	78.8 (4.1)	88.8 (9.9)	97.8 (23.2)	91.2 (6.9)	90.2 (4.7)	85.8 (7.4)
Creatinine	79.1 (28.0)	54.2 (6.9)	37.6 (7.7)	45.4 (7.5)	28.8 (4.9)	40.4 (11.5)
	80.4 (15.6)	56.0 (16.3)	25.6 (4.9)	39.2 (8.5)	25.2 (7.2)	41.4 (6.4)
Glucose	3.3 (1.9)	5.8 (2.1)	4.5 (3.1)	4.6 (2.5)	5.5 (1.7)	5.6 (3.9)
	9.2 (4.3)	7.1 (3.0)	5.2 (1.5)	5.6 (1.2)	3.7 (0.3)	6.3 (1.6)
Osmolarity	299 (10.9)	307 (25.5)	282 (15.6)	307 (20.1)	314 (15.6)	300 (15.7)
	301 (24.8)	315 (7.2)	294 (37.5)	306 (21.8)	302 (15.5)	289 (25.4)
Potassium	3.4 (1.3)	4.3 (1.2)	3.6 (1.1)	3.4 (0.8)	4.6 (1.4)	4.3 (1.4)
	3.3 (1.4)	3.9 (0.6)	3.7 (0.5)	3.9 (0.3)	3.6 (0.4)	4.4 (1.1)
Protein	25.4 (7.0)	31.8 (2.2)	33.2 (9.2)	32.6 (4.9)	43.4 (12.3)	36.8 (1.8)
	27.6 (8.3)	27.0 (7.2)	30.4 (10.8)	35.2 (4.4)	34.2 (6.6)	34.8 (4.5)
Sodium	153 (6.9)	155 (12.2)	143 (7.9)	154 (10.8)	158 (8.1)	153 (6.1)
	152 (10.3)	157 (6.9)	150 (19.2)	155 (11.5)	154 (8.3)	143 (11.7)
T_4	6.2 (3.5)	2.4 (1.9)	4.4 (2.6)	3.4 (3.3)	4.8 (2.6)	6.8 (2.7)
	3.8 (3.3)	1.8 (1.7)	6.0 (3.1)	6.0 (1.9)	4.0 (4.6)	2.8 (2.7)

[a] Controls followed by experimentally infected rainbow trout for each parameter.
[b] Mean followed by (standard deviation).

affect increased plasma cortisol in fish. This is because elevated plasma cortisol concentrations can increase the susceptibility of fish to infectious diseases (Pickering and Durston 1983; Pickering and Pottinger, 1985; Woo et al., 1987), probably because corticosteroids depress the inflammatory response, mobilization of leucocytes and phagocytosis (Grant, 1967). Claims that elevated concentrations of plasma cortisol increase the susceptibility of fishes to infectious diseases have been derived from experimental studies in which fish were injected with cortisol (Pickering and Durston, 1983; Pickering and Pottinger, 1985). However, as Laidley et al. (1988) aptly recognized, physiological changes associated with the natural disease process have been poorly studied. Our results that show there were no significant changes in the cortisol concentrations in blood of rainbow trout infected with *I. hoferi* support the study on *C. salmositica* (Laidley et al. 1988). Thus, while it is apparent in both Laidley et al. (1988) and this study that parasitemia constitutes a stressor, the lack of cortisol involvement in both disease processes suggests that cortisol elevation may not be a component of the host response to the natural disease process.

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Received for publication 19 June 1989.